THE USE OF CORTISONE AND OF LONG-ACTING ACTH TO ASSESS THE BIOLOGICAL ACTIVITY OF THE SOMATOTROPIC HORMONE (GROWTH HORMONE)

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The stimulating effect of the somatotropic hormone of the anterior lobe of the hypophysis in growth is based on its power to stimulate protein metabolism. As an anabolic hormone, somatotropin acts on every organ, which distinguishes it from the other tropic hormones of the hypophysis which have a selective action on a particular organ (a gland of internal secretion), but which makes its biological assessment difficult.

Many methods have been suggested for the biological assay of somatotropin. Some of these are based on the study of certain biochemical changes arising under the influence of the growth hormone (a fall in the non-protein nitrogen, urea, or amino acid content of the blood in dogs [13, 27, 28], a fall in the concentration of glutathione in the liver [16], an increase in the alkaline phosphatase or phosphorus content of the serum of hypophysectomized rats [23], retention of nitrogen [4, 14], an increase in the inclusion of sulfur from labled methionine in the skeletal muscles [11], etc).

Other methods are based on the stimulation of the body weight of normal rats in which the natural growth has been retarded and a so-called plateau established [10, 25], the stimulation of increase in weight of hypophysectomized rats [9, 24], the increase in the body weight of dwarf mice [12] and the increase in the length of the tail of hypophysectomized rats [8].

The most widely used method at the present time for the quantitative estimation of the biological activity of the somatotropic hormone is the "tibia test".

From examination of the development of the proximal epiphyseal cartilage of the tibia in normal and hypophysectomized rats it has been shown [21, 26] that after removal of the hypophysis the width of the cartilaginous epiphyseal plate is reduced and the equilibrium between the formation of cartilage and bone during endochondral ossification is disturbed. In the normal rat [5] the reduction in the thickness of the cartilaginous plate takes place mainly between the 40th and 65th day of life, and it subsequently proceeds at a slower rate. Removal of the hypophysis of 25-30 day-old rats leads to a rapid decrease in the width of the epiphyseal cartilage, and on the 14th day after operation this becomes constant. In response to injection of growth hormone an increase in the width of the cartilaginous epiphyseal plate of the tibia was observed in hypophysectomized rats [22]. On the basis of the data given above, a method of estimation of the biological activity of the growth hormone was developed, using 26-28 day-old female rats from which the hypophysis was removed [17]. From 12-14 days after hypophysectomy the rats were injected intraperitoneally for a period of 4 days with somatotropic hormone.

On the day after the last injection the rats were killed and, after suitable staining, the width of the epiphyseal cartilage of the tibia was measured under the microscope. The test was more sensitive than all

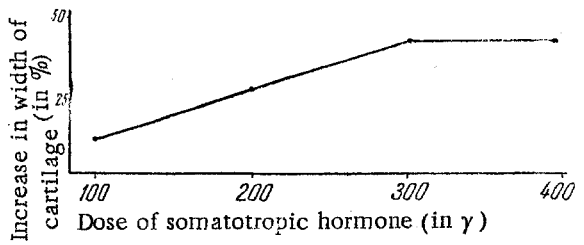

Fig. 1. Relationship between the increase in width of the epiphyseal cartilage of the tibia and the dose of somatotropic hormone.

other methods of determination of the biological activity of the growth hormone and did not take a long time (18 days).

It may, however, be carried out only on hypophysectomized animals, which introduces great difficulties in serial testing of the preparation, especially on a commercial scale. For this reason many workers have tried to find a method of determination of the biological activity of the somatotropic hormone in which hypophysectomy was unnecessary [1, 2, 7, 19].

The Effect of Methylthiouracil (30 mg per day) on the Body Weight and the Width of the Epiphyseal Cartilage

Duration of administration of MTU in days	Mean increase in body weight in g		Mean width of epiphyseal cartilage (in μ)	
	control	MTU	control	MTU
20	37	34	185	211
26	79	44	232	226
30	48	28	200	190
40	74	52	258	217
55	133	62	151	187
60	135	90	204	281
60	112	58	109	132
75	149	47	94	83

Our aim was to devise a method of testing the growth hormone in which the growth of the epiphyseal cartilage of the tibia could be inhibited without recourse to hypophysectomy. We took as our starting point reports in the literature and our own findings [3] that the action of ACTH and the corticosteroids was catabolic in character and that these preparations inhibited growth.

Becks, Simpson, Li and Evans [6] observed a reduction in the thickness of the epiphyseal cartilage after administration of ACTH. Huble [20] showed that cortisone markedly inhibits chondrogenesis in cockerels in the zones of proliferation (epiphyseal plate) of the proximal and distal ends of the femur, tibiotarsus and tarsometatarsus. Geschwind and Li [15] observed antagonism between hydrocortisone and the somatotropic hormone. After injecting 24 day-old female rats simultaneously with hydrocortisone and different doses of growth hormone, these authors observed marked inhibition of the increase in width of the epiphyseal cartilage of the tibia compared with that taking place in normal animals in response to somatotropin.

Our aim was to find out, in the first place, whether it was possible by the use of cortisone or ACTH to inhibit chondrogenesis in the epiphyseal cartilage of the tibia in female rats, and to obtain in a short period of time a reduction in the width of the epiphyseal cartilage of roughly the same degree as after hypophysectomy, and in the second place, whether in such rats stimulation of chrondrogenesis takes place in the epiphyseal cartilage of the tibia under the influence of growth hormone.

EXPERIMENTAL METHOD AND RESULTS

The effect of cartisone was investigated in 120 female rats weighing 30-35 g. On each of four successive days, 85 rats were injected with 1.5-2.0 mg of cortisone, and 35 rats received 0.05 mg daily for 14 days. The cortisone was injected once a day, subcutaneously. In all the experiments the cortisone inhibited the prolifera-

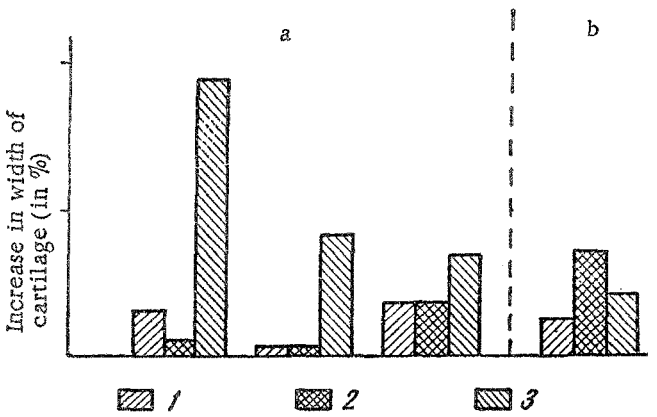

Fig. 2. The effect of combined injection of somatotropic and thyrotropic hormones on the width of the epiphyseal cartilage of the tibia.
a) Administration of thyrotropic hormone in a dose of 400 γ; b) administration of throtropic hormone in a dose of 75 γ; 1) thyrotropic hormone; 2) somatotropic hormone; 3) somatotropic and thyrotropic hormones.

ation of the epiphyseal cartilage of the tibia and caused a reduction in its width. When 1.5 mg of cortisone* was injected daily for 4 days, the reduction in the width of the cartilaginous plate was 92-302 μ, or 23-63% of the control values, with an average of 41%. Injection of 0.5 mg of cortisone daily for 14 days caused a reduction in the width of the epiphyseal cartilage by 23-147 μ, or 8-39 % of the control values, an average of 26%. The first method of injection of cortisone thus caused a more marked reduction in the width of the cartilaginous plate of the tibial epiphysis than the second. We compared the effect of injection of cortisone with that of removal of the hypophysis. The reduction in the width of the cartilaginous plate on the 14th day after hypophysectomy was on the average 49%, and after daily injection of 1.5 mg of cortisone for 4 days, it was 41%.

A similar although weaker effect was observed after the use of ACTH-zinc phosphate, i.e. a long-acting preparation (10-20 units daily for 4 days).

The width of the cartilaginous plate was reduced by 72-207 μ, i.e. by 21-44% of the control values, on the average by 32% (results for 45 rats). It must be emphasized that ACTH of the ordinary variety of action had no effect on the proliferation of the epiphyseal cartilage of the tibia, which was probably due to the fact that this preparation was rapidly excreted from the body.

We had at our disposal several samples of somatotropic hormone (STH), prepared in biochemical and experimental production laboratories of the All-Union Institute of Experimental Endocrinology, which contained insignificant traces of thyrotropin (less than 75 γ/mg STH). When injected intraperitoneally in a daily dose of 75 and 100 γ for 4 days to 100 intact rats, in which proliferation of the epiphyseal cartilage was inhibited by cortisone, STH stimulated growth of this cartilage and increased the width of the epiphyseal plate by 49-135 μ (400 γ STH) and by 57-187 μ (300 γ STH), i.e. on the average by 87 μ. The width of the epiphyseal cartilage was thus restored on the average by 50%. Similar results were obtained by the action of STH on 30 rats receiving ACTH-zinc phosphate. The results obtained were compared with the results following administration of a highly purified preparation of growth hormone, standardized in international units. 300-400 γ of such a highly purified STH also stimulated growth of the epiphyseal cartilage of the tibia on the average by 50% compared with control animals receiving cortisone.

We investigated the effect of various doses of STH from 100 to 800 γ (total dose) on the increase in width of the epiphyseal cartilage. With an increase in the dose of STH from 100 to 300 γ, a direct relationship was observed between the increase in width of the cartilaginous plate of the tibia in rats receiving cortisone and the dose of STH. Increasing the dose to 400γ and above was not accompanied by any further increase in the growth of the cartilage (Fig. 1).

On the basis of the results obtained we considered it possible to use intact female rats, weighing 30-35 g, for the biological assay of growth hormone, by replacing the complicated operation of hypophysectomy by injection of cortisone or ACTH-zinc phosphate according to the method described as follows.

Cortisone is injected subcutaneously in a single daily dose of 1.5-2.0 mg for 4 days. After the injections of cortisone have come to an end, STH is injected intraperitoneally in a daily dose of 75 or 100 γ for 4 days. This daily dose is given as three separate injections. The total dose of STH is 300-400 γ. 24 hours after the last injection of STH (the 9th day after the start of the test) the animals are killed and one or both tibias are removed, freed from soft tissues, split longitudinally and quickly stained or fixed in 10% formalin, the further treatment of the bone being according to the method described by Greenspan, Li, Simpson and Evans [17, 18].

* Cortisone from the firms of Merck, Roussel and Biddle Sawyer.

Before staining, the specimens are washed in water for half an hour, transferred to acetone for 1 hour and then again washed in water for half an hour. The bone is next placed in a freshly prepared 2% solution of silver nitrate for 1½-2 minutes, rinsed in water, and exposed in water to the action of sunlight or illuminated with a clear electric lamp until the calcified areas stain a dark brown color. The stained halves of the bone are immersed for 25-30 seconds in 10% hyposulfite, rinsed in tap water for half an hour and allowed to stand in 80% ethyl alcohol in the dark. The width of the cartilaginous plate of the proximal epiphysis is measured by means of an ocular micrometer under low-power magnification of the microscope. Not less than 10 measurements are made of each half of the bone, and these values are converted into microns. Where cortisone is unobtainable it may be replaced by ACTH-zinc phosphate (10-20 units once a day, subcutaneously, for 4 days).*

We also investigated the possibility of using methylthiouracil (MTU) as an inhibitor of growth of the epiphyseal cartilage of the tibia [1]. In 8 series of experiments we gave 30 mg of MTU daily with the diet for 20-75 days. Besides complete failure of the animals to gain weight, or even loss of weight, in accordance with the reports in the literature [1, 2], we observed that the width of the epiphyseal cartilage was small. When we compared the width of the epiphyseal cartilage in 80 rats receiving MTU and 80 control animals of the same age, however, we found that the reduction in the width of the epiphyseal cartilage was not only the result of the action of MTU but also was in consequence of the retardation of growth due to age. In control animals not receiving MTU, the cartilaginous plate was roughly of the same width as in animals of the same age exposed to the action of MTU (see Table).

The failure of the animals receiving MTU to gain weight, associated with absence of changes in chondrogenesis in the tibial epiphysis, is probably evidence of the reduction in body weight of the rats in which the thyroid gland was blocked chemically being due not only to inhibition of true growth but also to disturbance of metabolism caused by exclusion of the function of the thyroid gland. Injection of STH in a total dose of 400 γ to rats both receiving and not receiving MTU increased the width of the epiphyseal cartilage on the average by 40 μ.

In conclusion we consider it necessary to draw attention to the importance of contamination of the STH preparation with thyrotropic hormone. From our observations, which are in agreement with findings in the literature, thyrotropic hormone in a dose of 400 γ has an insignificant effect on the proliferation of the epiphyseal cartilage (19 μ). However, after the simultaneous injection of 400 γ of thyrotropic hormone and 100 γ of STH (i.e., an ineffective dose), a significant increase in the width of the cartilaginous plate of the proximal tibial epiphysis was observed (Fig. 2). In other words, synergism exists between the thyrotropic and somatotropic hormones, which interferes with the objective assay of the growth hormone by means of the tibial test. After the simultaneous injection of small doses of thyrotropic hormone (75 γ) with STH, no synergism was observed in relation to the growth effect. For a correct estimation of the biological activity of STH, it is therefore essential to make allowance for the content of thyrotropic hormone therein. Only when the contamination by the latter is insignificant (75 γ/mg STH) can the action of the growth hormone, qualitative and quantitative, be objectively assessed.

SUMMARY

A method of evaluating the biological effect of the somatotropic hormone is given. It is based on the ability of the growth hormone to stimulate the proliferation of the epiphyseal cartilage of rat's tibia. The inhibition of the proliferation of the epiphyseal cartilage is reached by the subcutaneous administration of cortisone for 4 days (1.5 mg once a day). Subsequent injections of the somatotropic hormone (75-100γ daily for 4 days) reestablish the width of the epiphyseal cartilage to 50% on the average. In the absence of cortisone it may be replaced by ACTH-zinc-phosphate (10 units subcutaneously once a day for 4 days). The usual ACTH cannot be employed for this purpose.

* When our research had been completed, the abstracting journal "Biologiya" [No. 6, 1957] published an abstract No. 24314 of the paper by P. Sforzini, M. Negri and A. Mazzarella, from which it was gathered that these workers had successfully used large doses of ACTH to suppress growth of the epiphyseal cartilage of the tibia and had subsequently stimulated growth by means of growth hormone.

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